Published online: August 25, 2017

News & Views

THE EMBO JOURNAL

RNA polymerase I, bending the rules?

Laura Jochem, Ewan P Ramsay & Alessandro Vannini

Transcription initiation is one of the key regulatory steps in expressing the genetic information encoded in the DNA. Mechanisms of RNA Pol II transcription have been extensively studied, whereas the structural basis of RNA Pol I and III transcription is still poorly defined. Three recent studies discussed here give a first glimpse into the molecular mechanisms underlying the process of RNA Pol I transcriptional initiation and reveal unexpected structural differences compared to the previously described homologous Pol II structures.

See also: **Y Sadian *et al*** and **Y Han *et al*** (June 2017) and **C Engel *et al*** (March 2017)

In eukaryotes, transcription requires three RNA polymerase (Pol) enzymes, Pol I, II and III, which all contain a 10 subunit conserved core and active centre (Cramer *et al*, 2008). The 14 subunit RNA Pol I accounts for 60% of total nuclear transcription through synthesis of the large rRNA precursor, which serves as a building block for ribosome biogenesis.

Transcriptional initiation requires the assembly of a pre-initiation complex (PIC), which is formed when the RNA polymerase is recruited to its target genes by a set of specific general transcription factors (GTFs). The GTFs required for this process are distinct for each RNA polymerase, but the overall positioning and general function of related GTFs are thought to be conserved (Vannini & Cramer, 2012). A central role in forming the RNA Pol PICs is performed by TFIIB and TFIIB-like factors, bridging GTF complexes and RNA Pol II (Vannini & Cramer, 2012).

During transcriptional initiation, the PIC forms multiple functional states known as the closed complex (CC), the open complex (OC) and the initially transcribing complex (ITC), which then transitions into the elongating complex (EC). Firstly, the PIC CC binds closed double-stranded promoter DNA. Transition into the OC occurs through the melting of the DNA and insertion of the template strand DNA into the active site. RNA synthesis starts with initially forming an ITC bound to the GTFs, which is followed by dissociation of the GTFs and the formation of an EC. Recent advances in cryo-electron microscopy (EM) have been exploited to visualize this dynamic process at an unprecedented level of detail, and high-resolution structures of different functional states of the RNA Pol II PIC have been reported (reviewed in Nogales *et al*, 2017).

Currently, there is still a lack of information about the exact molecular mechanism underlying Pol I transcription initiation. In yeast, RNA Pol I PIC formation requires Rrn3 and the Core Factor (CF), consisting of Rrn6, Rrn11 and Rrn7, the latter being the Pol I-specific TFIIB-like factor. In its inactive form, RNA Pol I exists as a dimer and upon binding to Rrn3, Pol I is stabilized in its monomeric form and can bind to the CF via Rrn3 (Blattner *et al*, 2011; Bedwell *et al*, 2012; Engel *et al*, 2013; Fernández-Tornero *et al*, 2013; Neyer *et al*, 2016; Tafur *et al*, 2016). Previously, models for the Pol I PIC had been proposed based on the structural and functional conservation between the core transcription initiation complexes (Vannini & Cramer, 2012; Knutson *et al*, 2014).

Recently, a series of studies employed a structural approach to characterize Pol I transcription initiation at a molecular level (Engel *et al*, 2017; Han *et al*, 2017; Sadian *et al*, 2017). All three studies exploited cryo-EM to investigate the Pol I PIC, revealing an unexpectedly different structure to the homologous Pol II PIC. A striking feature observed in all three studies is an unexpected arrangement of the CF, with implications for DNA recognition and polymerase recruitment. Interestingly, despite the homology shared between Rrn7 and TFIIB, Rrn7 adopts an altered arrangement in the Pol I PIC compared to the Pol II assembly. The Rrn7 cyclin domains, important mediators of the TFIIB-Pol II interaction, do not contact Pol I. This represents a dramatic difference in the DNA recognition of the Pol I machinery compared to Pol II. Rrn7 and Rrn11 interactions with the backbone of the DNA induce, surprisingly, a bend of approximately 30° in the upstream DNA compared to the sharp 90° bend induced by TBP-TFIIB in the Pol II machinery. Notably, although TBP is present in the biochemical preparation of the Pol I PICs, no density could be attributed to it, implying disorder or instability of this subunit. The observed bending threads the DNA between, instead of over, the Pol I wall and protrusion, which directly contacts the DNA. The tight arrangement of the DNA leads Engel *et al* (2017) to suggest that the Pol I PIC produces a "gate" into which only promoters capable of adopting the required deformation are engaged, thus recognizing the biophysical features rather than the promoter sequence. Indeed, this is consistent with the lack of sequence conservation in Pol I promoters. Additionally, the unusual threading more closely resembles the EC, suggesting a complex adapted for rapid promoter escape and high transcription efficiency.

Comparing the three studies also displayed some interesting differences. Despite the presence of Rrn3 in the OC and ITC resolved by Engel *et al* (2017) and Sadian *et al* (2017), respectively Han *et al* (2017), report its loss, suggesting that the subunit dissociates upon formation of the ITC. Additionally, despite being used for

The Institute of Cancer Research, London, UK. E-mail: alessandro.vannini@icr.ac.uk
DOI 10.15252/embj.201797924

the assembly of the PIC, the RNA product was not visible in the reconstructions from Sadian *et al* (2017) leading the authors to describe this complex as a pre-active Pol I OC. The reconstructions from Han *et al* (2017) show different states of the opening of the cleft and competition between the Rrn3 Zn-ribbon and the A49 tandem winged helix domain. These differences highlight the dynamics of the Pol I PIC complex, yet might also be explained in the light of the different nucleic acid scaffolds used and/or the different biochemical isolation of Pol I PICs. In fact, Han *et al* (2017) purified the complex using affinity capture of labelled DNA compared to dialysis and gel filtration approaches employed by Engel *et al* (2017) and Sadian *et al* (2017), respectively.

Taken together, the three studies give an unprecedented insight into the mechanism of Pol I transcription initiation (Fig 1). Pol I recruitment by CF is followed by DNA threading through the wall and protrusion "gate" to the active site. Bending of the DNA, followed by the engagement of the Rrn7 zinc-ribbon and progressive closure of the active site cleft, might cause

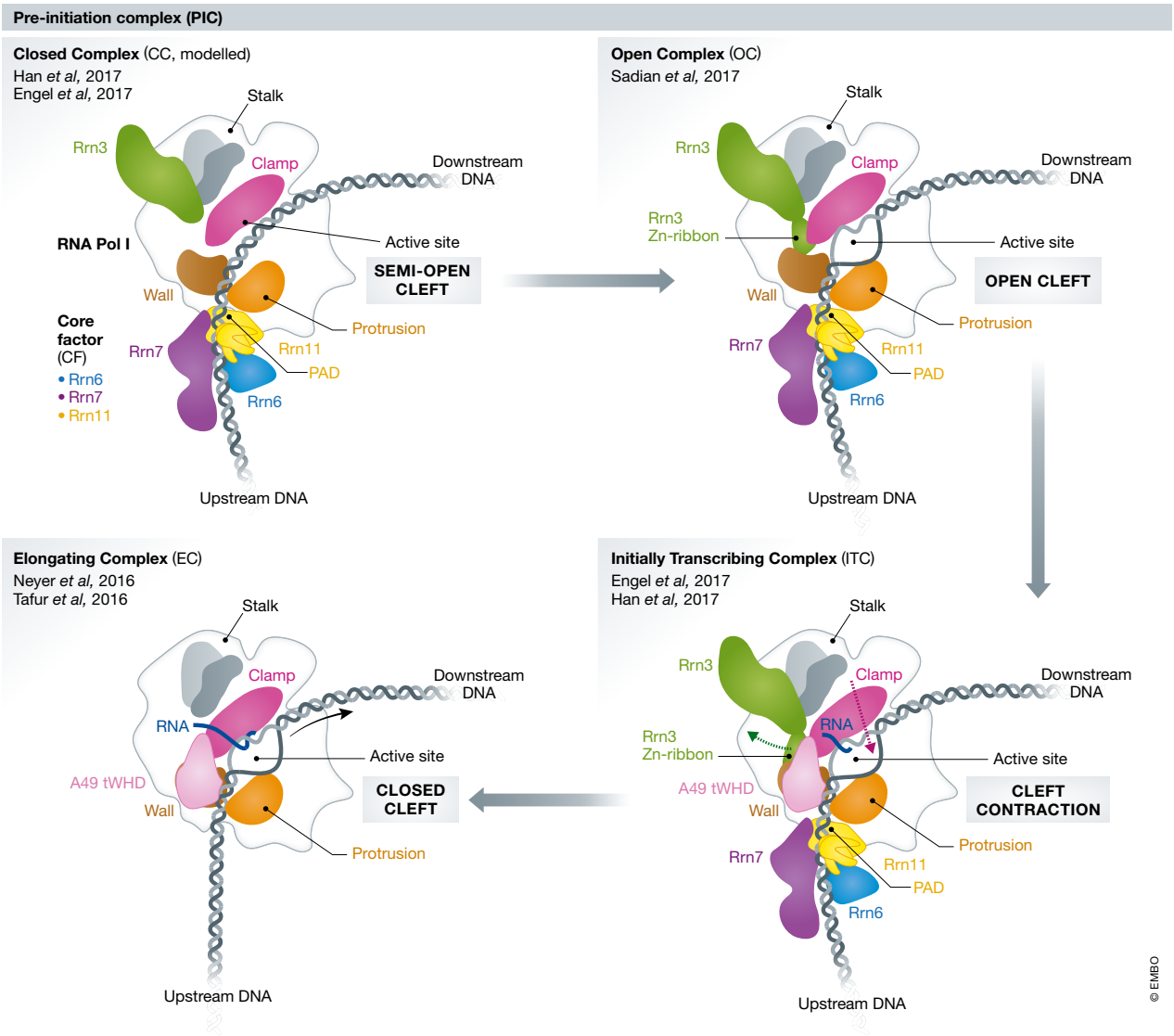

Figure 1. Schematic of RNA Pol I transcriptional initiation.
The general positioning of Rrn3 (green) and CF (Rrn7, purple, Rrn6, blue, Rrn11, yellow) is shown. Upon PIC formation, CF-bound DNA is inserted into Rrn3-Pol I, threaded between the wall (in brown) and protrusion (in orange) and bent by 30° (CC). The DNA is subsequently melted and a transcription bubble forms, stabilized by the Rrn3 Zn-ribbon (OC). During the transition from the OC to the ITC, the cleft is progressively contracted and the Rrn3 Zn-ribbon partially displaced by the tandem winged helix domain of subunit A49 (A49 tWHD, in light pink). Dissociation of the GTFs, narrowing down of the clamp and complete disengagement of the Rrn3 Zn-ribbon lead to promoter escape and the formation of a closed EC.

spontaneous opening of the rDNA promoter, causing the transition from a CC to an OC PIC. In this process, the DNA sequence itself plays an important role, as it has been noted that only sequences characterized by a certain degree of malleability could be efficiently unzipped by the minimal Pol I PIC (Engel *et al*, 2017). Upon synthesis of short nascent RNA, the OC then transits into an ITC. The ITC is characterized by further contraction of the cleft and the progressive ordering of the tandem winged helix domain of subunit A49, which partially displaces the Rrn3 Zn-ribbon. Formation of the ITC then allows for full elongation of the rRNA transcript and promoter clearance, completing initiation.

In summary, the combinatorial approaches of modern cryo-EM, X-ray crystallography, and *in vitro* assays employed across the studies allowed for a fine dissection of the mechanism of Pol I transcription initiation. The three complementary studies revealed an unexpected arrangement and unusual bending of the upstream DNA, highlighting the complexity of the transitions occurring during the transcription initiation cycle. Further studies will be needed to understand how TBP and the upstream factor UAF contributes towards specific and efficient RNA Pol I initiation. Taking into account the well-documented role of Pol I transcription in cancer, such information may be important for the development of anti-cancer therapeutics targeting the Pol I machinery.

As a final note, we would like to highlight that solving the crystal structure of the CF in the study by Engel *et al* (2017) allowed the authors to build a very accurate three-dimensional model, aiding the interpretation of EM maps and the design of mutants for biochemical experiments. Thus, in the era of the cryo-EM "resolution revolution", it appears that X-ray crystallography still has a very prominent place in the structural biologists' toolbox.

References

Bedwell GJ, Appling FD, Anderson SJ, Schneider DA (2012) Efficient transcription by RNA polymerase I using recombinant core factor. *Gene* 492: 94 – 99

Blattner C, Jennebach S, Herzog F, Mayer A, Cheung ACM, Witte G, Lorenzen K, Hopfner K-P, Heck AJR, Aebersold R, Cramer P (2011) Molecular basis of Rrn3-regulated RNA polymerase I initiation and cell growth. *Genes Dev* 25: 2093 – 2105

Cramer P, Armache K-J, Baumli S, Benkert S, Brueckner E, Buchen C, Damsma GE, Dengl S, Geiger SR, Jaslak AJ, Jawhari A, Jennebach S, Kamenski T, Kettenberger H, Kuhn C-D, Lehmann E, Leike K, Sydow JE, Vannini A (2008) Structure of eukaryotic RNA polymerases. *Annu Rev Biophys* 37: 337 – 352

Engel C, Sainsbury S, Cheung AC, Kostrewa D, Cramer P (2013) RNA polymerase I structure and transcription regulation. *Nature* 502: 650 – 655

Engel C, Gubbey T, Neyer S, Sainsbury S, Oberthuer C, Baejen C, Bernecky C, Cramer P (2017) Structural basis of RNA polymerase I transcription initiation. *Cell* 169: 120 – 131.e22

Fernández-Tornero C, Moreno-Morcillo M, Rashid UJ, Taylor NMI, Ruiz FM, Gruene T, Legrand P, Steuerwald U, Müller CW (2013) Crystal structure of the 14-subunit RNA polymerase I. *Nature* 502: 644 – 649

Han Y, Yan C, Nguyen THD, Jackobel AJ, Ivanov I, Knutson BA, He Y (2017) Structural mechanism of ATP-independent transcription initiation by RNA polymerase I. *Elife* 6: e27414

Knutson BA, Luo J, Ranish J, Hahn S (2014) Architecture of the *Saccharomyces cerevisiae* RNA polymerase I Core Factor complex. *Nat Struct Mol Biol* 21: 810 – 816

Neyer S, Kunz M, Geiss C, Hantsche M, Hodirnau VV, Seybert A, Engel C, Scheffer MP, Cramer P, Frangakis AS (2016) Structure of RNA polymerase I transcribing ribosomal DNA genes. *Nature* 540: 607 – 610

Nogales E, Louder RK, He Y (2017) Structural insights into the eukaryotic transcription initiation machinery. *Annu Rev Biophys* 46: 59 – 83

Sadian Y, Tafur L, Kosinski J, Jakobi AJ, Wetzel R, Buczak K, Hagen WJH, Beck M, Sachse C, Müller CW (2017) Structural insights into transcription initiation by yeast RNA polymerase I. *EMBO J* https://doi.org/10.15252/embj.201796958

Tafur L, Sadian Y, Hoffmann NA, Jakobi AJ, Wetzel R, Hagen WJ, Sachse C, Muller CW (2016) Molecular structures of transcribing RNA polymerase I. *Mol Cell* 64: 1135 – 1143

Vannini A, Cramer P (2012) Conservation between the RNA polymerase I, II, and III transcription initiation machineries. *Mol Cell* 45: 439 – 446